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Two Patients With Duplication of 17p11.2: The Reciprocal of the Smith-Magenis Syndrome Deletion?

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J.M. and H.G. are two unrelated male patients with developmental delay. Cytogenetic analysis detected a duplication of 17p11.2 in both patients. The extent of the duplicated region was determined using single copy DNA probes: cen-D17S58-D17S29-D17S258-D17S71-D17S445-D17S122-tel. Four of the six markers, D17S29, D17S258,D17S71, and D17S445, were duplicated by dosage analysis. Fluorescent in situ hybridization (FISH) analysis of H.G., using cosmids for locus D17S29, confirmed the duplication in 17p11.2. Because the deletion that causes the Smith-Magenis syndrome involves the same region of 17p11.2 as the duplication in these patients, the mechanism may be similar to that proposed for the reciprocal deletion/duplication event observed in Hereditary Neuropathy with Liability to Pressure Palsies (HNPP) and Charcot-Marie-Tooth Type 1A disease (CMT1A). © 1996 Wiley-Liss, Inc.

KEY WORDS: duplication 17p11.2, Smith-Magenis syndrome, deletion 17p11.2

INTRODUCTION

Trisomy for the short arm of chromosome 17 was first reported in a patient with multiple malformations and a small supernumerary chromosome comprised of 17pter→17q11 [Latta et al., 1974]. There have been several subsequent reports of patients with duplication or partial trisomy for 17p. A number of these cases have involved duplication of the entire short arm of the chromosome, while others have been partial duplications involving only the proximal short arm [Palutke et al., 1976; Bartsch-Sandhoff et al., 1979; Shabtai et al., 1979; Feldman et al., 1982; Jinno et al., 1982; Mascarello et al., 1983; Docherty et al., 1983; Magenis et al., 1986; Schrander-Stumpel et al., 1990; Kozma et al., 1991; Friedman et al., 1992; Lupski et al., 1992; Upadhyaya et al., 1993]. Features commonly observed in duplication 17p are pre- and postnatal growth retardation, severe development delay, microcephaly, and facial dysmorphism including downslanting palpebral fissures, hypertelorism, long philtrum, thin upper lip, and micrognathia [Latta et al., 1974; Palutke et al., 1976; Bartsch-Sandhoff et al., 1979; Shabtai et al., 1979; Feldman et al., 1982; Jinno et al., 1982; Mascarello et al., 1983; Schrander-Stumpel et al., 1990]. The phenotypic similarity observed among patients had led authors to suggest the existence of a "trisomy 17p syndrome" [Jinno et al., 1982; Mascarello et al., 1983; Schrander-Stumpel et al., 1990].

There have also been reports of cytogenetically visible duplications of 17p in individuals with Charcot-Marie-Tooth 1A syndrome [Lupski et al., 1992; Chance et al., 1992; Upadhyaya et al., 1993]. CMT1A is the most common of the inherited peripheral neuropathies and is usually associated with a submicroscopic duplication of a 1.5 Mb region in 17p11.2→p12 [Lupski et al., 1991]. In addition, there have been two clinical reports with cytogenetically visible duplications involving 17p11.2→17p12 but without the CMT1A phenotype [Magenis et al., 1986; Kozma et al., 1991; Greenberg et al., 1992]. These latter duplications apparently did not extend into the CMT1A critical region.

Most of the visible duplications of 17p have not been subjected to molecular analyses; therefore the extent of the duplications have not been defined. In the present report, we describe two patients with duplications involving only 17p11.2. This is the same chromosomal region which is deleted in patients with the Smith-Magenis syndrome (SMS). Cytogenetic and molecular studies including Southern blot analysis and fluorescent in situ hybridization (FISH) were performed to characterize these duplications. The findings suggest that the duplications observed in our patients may represent the reciprocal of the 17p11.2 deletion seen in SMS.

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MATERIALS AND METHODS

Case Reports

J.M. is a 5-year-old white male born to a 24-year-old gravida 2 female. During the neonatal period, J.M. was noted to have a grade II/VI heart murmur which was no longer detectable by 4 months of age. At 9 months of age, J.M. was evaluated for developmental delay and failure to thrive. His weight and head circumference were less than the third centile and his height was at the tenth centile. He had a flat nasal bridge, slight epicanthal folds, and narrow palpebral fissures. His speech and language skills were below normal.

H.G. is a white male born to a 21-year-old gravida 3 mother. A previous pregnancy had resulted in a child who died at age 10 days following heart surgery for pulmonary artery atresia. At 2 days of age, H.G. was noted to have mild jaundice, decreased muscle tone, borderline low-set ears, and bilateral preaurcular pits. He was a slow feeder. At a follow-up evaluation at age 3 months, he presented with poor weight gain and a deficit in visual and auditory acuity. Face appeared normal.

Cytogenetic Analysis

Chromosome analysis was performed on peripheral blood lymphocytes from J.M. and his parents. Lymphocytes were cultured for 72 hours at 37°C in tissue culture medium RPMI 1640 with 20% fetal calf serum. Cultures were treated with ethidium bromide prior to harvest, according to the method of Ikeuchi [Ikeuchi et al., 1984]. Chromosome preparations were stained by the trypsin-Giemsa method [Patil et al., 1971].

For H.G., amniocentesis was performed at 17 weeks gestation due to increased risk of trisomy 21 based on maternal serum screening. Amniocytes were cultured in Chang D medium for 6 days at 37°C, harvested by standard techniques, and G-banded by the trypsin-Giemsa method [Patil et al., 1971]. Cytogenetic analysis of the parents was performed on chromosome spreads from peripheral blood lymphocytes cultured in medium RPMI 1640 with 20% fetal calf serum. Following birth, a peripheral blood sample was obtained from the patient for confirmatory cytogenetic studies. Chromosome elongation was achieved using a combination of synchronization and ethidium bromide treatment [Lawce et al., 1991].

Fluorescent In Situ Hybridization

Metaphase spreads were prepared from amniocytes and hybridized with the digoxigenin-labeled Smith-Magenis Chromosome Region (SMCR) probe provided in the Oncor Kit P5154-DIG. This probe contains cloned DNA specific sequences at the locus D17S29 in 17p11.2 as well as cloned material specific to a locus (RARA) in 17q21.1 that aids in the identification of the chromosome 17 homologs. Slide denaturation, hybridization, and post-hybridization washes were performed according to the procedure from Oncor.

Molecular Analysis

Genomic DNA from the patients and their parents was prepared from peripheral blood by a method described previously [Schwartz et al., 1990]. Five micrograms of DNA was digested with various restriction endonucleases according to the supplier's recommendations and the fragments were separated on a 0.7% agarose gel, transferred to Hybond N+ (Amersham, Arlington Heights, IL) nylon membrane, and subsequently prehybridized and hybridized according to the manufacturer's protocol. After an overnight hybridization, the filters were washed to a stringency of 0.1 × SSC, 1% SDS. Filters were exposed to Kodak X-ray film from 1 to 6 days at −70°C. The DNA probes used in this analysis were labeled by random primer extension [Feinberg et al., 1983]. The dosage of each marker was determined by using a Molecular Dynamics (Model 300A) scanning laser densitometer as described previously [Schwartz et al., 1988]. In brief, an autosomal probe A1(D4S12) was hybridized to the filter at the same time as the chromosome 17 marker. For each allele band, the area under the peak was calculated. To estimate the number of copies of the marker allele in question, its peak area was divided by the peak area for D4S12. (A1 gives a single band for all enzymes except HaeIII.) This number was then divided by a similar number derived by averaging data obtained from three control individuals on the same filter.

RESULTS

Cytogenetic Evaluation

Cytogenetic analysis of J.M. showed extra material in the proximal short arm of chromosome 17 representing an apparent duplication of 17p11.2 (Fig. 1). His karyotype was designated 46,XY,dup(17)(p11.2p11.2). Parental chromosomes were normal.

Prenatal chromosome studies on H.G. revealed extra material in the proximal short arm of chromosome 17 representing an apparent duplication of 17p11.2. Parental karyotypes were normal. Follow-up studies on peripheral blood obtained postnatally from H.G. confirmed the finding of an abnormality involving the proximal short arm of chromosome 17. Based on the G-banding pattern, the abnormality was interpreted as a duplication of the region 17p11.2 (Fig. 1). Karyotype designation was 46,XY,dup(17)(p11.2p11.2).

FISH Evaluation

FISH analysis using cosmids for the locus D17S29 on metaphase spreads of H.G. revealed a more intense signal in the region of 17p11.2 on one of the chromosome 17 homologues (Fig. 2). The increased intensity was observed in 20/20 spreads examined from H.G.

Molecular Analysis

Six DNA markers localized to 17p11.2 were used to perform the analysis of the duplications present in the two patients [Wright et al., 1990; Greenberg et al., 1991]. The order of the markers on chromosome 17 is cen-D17S58-D17S29-D17S258-D17S71-D17S445-D17S122-tel. Densitometric studies detected two copies of the DNA markers D17S58 and D17S122 in each patient and three copies of the markers D17S29, D17S258, D17S71, and D17S445 (Table I). Therefore, the most centromeric and telomeric markers, D17S58 and D17S122, respectively, define the boundaries of the duplicated regions in these two patients (Fig. 3). For patient H.G., the duplication appeared to be of mater-

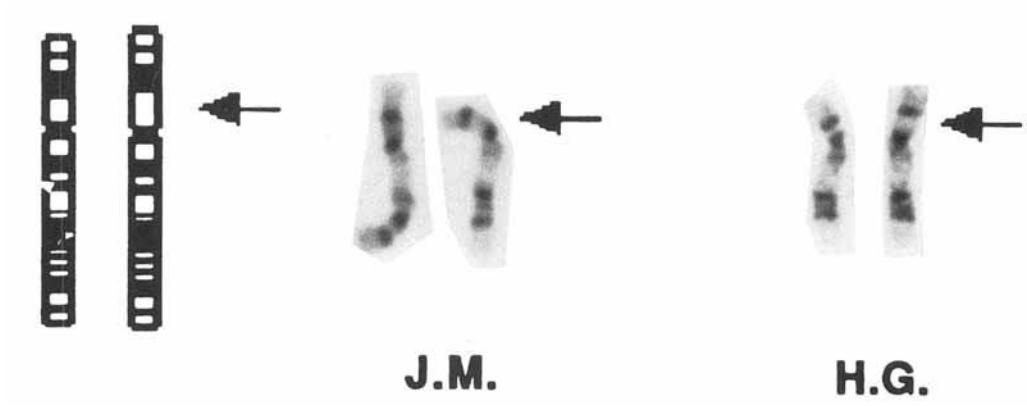

Fig. 1. Partial karyotype of patients J.M. and H.G., showing the chromosome 17 homologs. Duplication of 17p11.2 is indicated by the arrows.

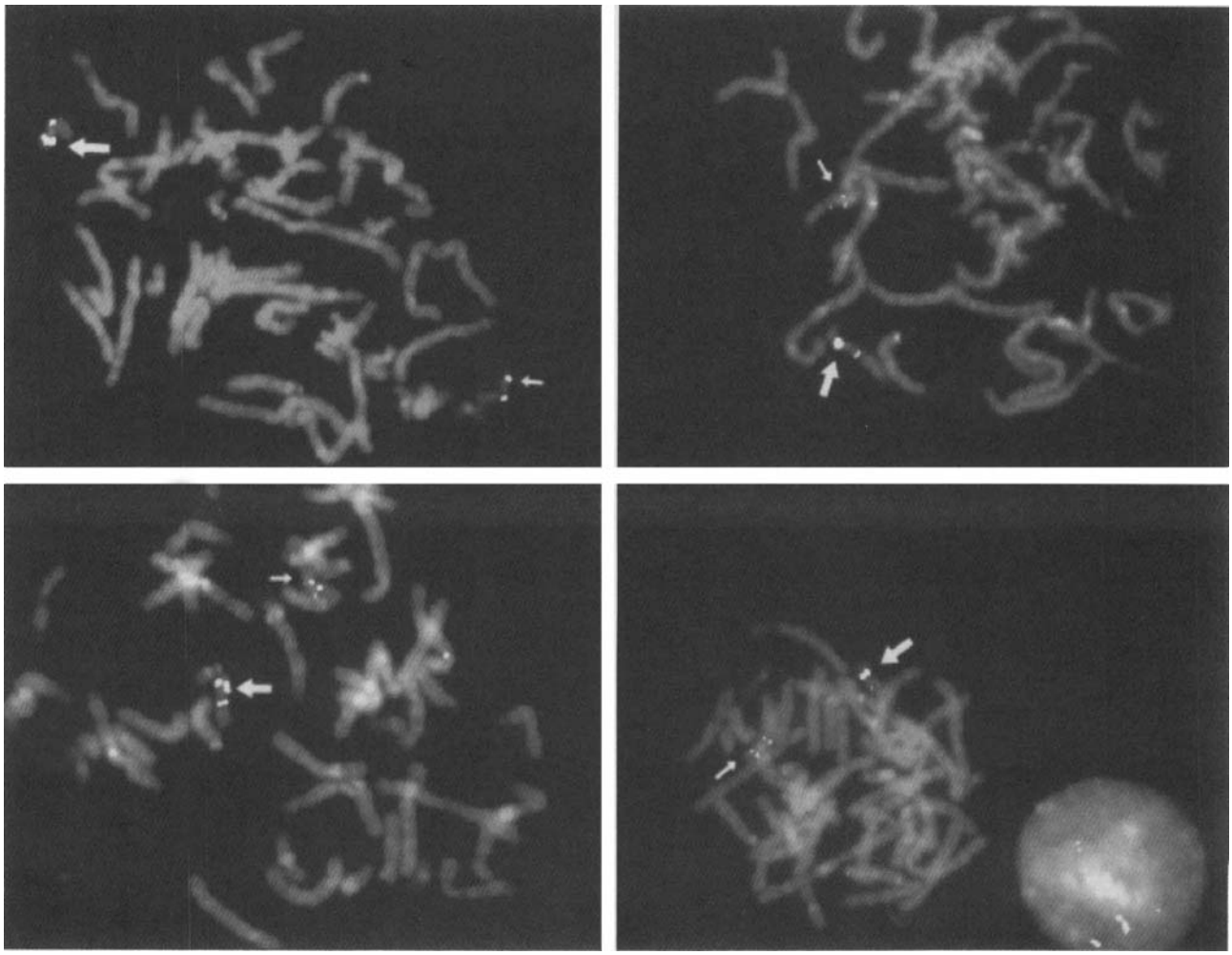

Fig. 2. Four representative FISH results on patient H.G. with the SMCR probe. The control probe (small arrow) shows equally intense signals on both chromosome 17 homologs while the SMCR probe shows a more intense signal on the affected chromosome (large arrow).

TABLE I. Summary of Analysis Using Markers Localized in 17p11.2

	Scoring of alleles[a]					
Marker	F	J.M.	M	F	H.G.	M
D17S58	1,2	1 (2.0)	1,2	1,2	2 (1.7)	2,2
D17S29	1,1	1 (2.9)	1,1	1,1	1 (1.3)	1,2
					2 (2.5)	
D17S258	1,2	1 (2.5)	1,1	2,2	2 (3.1)	2,2
		2 (0.9)				
D17S71	2,2	2 (3.3)	2,2	1,1	1 (0.8)	2,2
					2 (2.4)	
D17S445	1,2	1 (2.3)	1,1	1,1	1 (3.1)	1,1
		2 (1.4)				
D17S122	1,1	1 (0.9)	1,2	1,2	2 (2.1)	2,2
		2 (0.8)				

[a] Numbers in parentheses reflect the relative dosage of band present on autoradiograph. Two copies would be equivalent to 2.0. The number was calculated as described in Materials and Methods. F, father; M, mother.

nal origin based on the inheritance of alleles with the markers D17S29 and D17S71. With patient J.M., the parental origin could not be determined from our data.

DISCUSSION

We report two patients with cytogenetically visible expansions of 17p11.2. Molecular analysis determined that the duplication included four DNA markers: cen-D17S29-D17S258-D17S71-D17S445-tel. The duplicated region was flanked by the markers D17S58 and D17S122. As all of these markers are confined to 17p11.2, the molecular findings are consistent with the cytogenetic observation that no portion of the dark band 17p12 is present in the duplicated region (Fig. 1).

Two patients with apparently similar duplications have been reported by Magenis et al. [1986] and Kozma et al. [1991]. The cytogenetic interpretations of these earlier cases indicated that the duplications extended into 17p12. Molecular confirmation of the extent of the presumed duplications has not been reported. Neurological evaluation of these patients indicated they had normal nerve conduction velocities suggesting that the duplications did not in fact extend into 17p12 [Greenberg et al., 1992]. In addition to developmental delay, the clinical findings of the patients of Magenis et al. [1986] and Kozma et al. [1991] included facial anomalies, abnormal ears, club foot, and downslanted palpebral fissures. Kozma et al. [1991] also reported that their patient had maxillary hypoplasia, high arched palate, café-au-lait spots, and a short sternum. The less severe phenotypes observed in our two patients may be due to smaller amounts of duplicated material.

The region of duplication in our patients is equivalent to the deleted region in many Smith-Magenis syndrome patients [Greenberg et al., 1991]. Based on the analysis of DNA markers, the duplication in H.G. appears to arise from unequal sister chromatid exchange involving the maternal chromosome 17. The parental origin of the duplication in J.M. could not be determined nor could the mechanism leading to his duplication. Based on the analysis of chromosome 17 markers in J.M., his duplication could have resulted from unequal crossover between paternal homologs, unequal crossover between maternal homologs, or sister chromatid exchange on a maternal chromosome 17. Identification of more informative markers may help define the origin of the duplication in J.M.

Because the origin of the duplication in J.M. was not determined, it remains possible that the duplications in our two patients are of different parental origin, i.e., H.G.'s duplication involves the maternal chromosome 17, while J.M.'s duplication may be of paternal origin. Furthermore, the duplications in these two patients may have arisen from the same mechanism (unequal

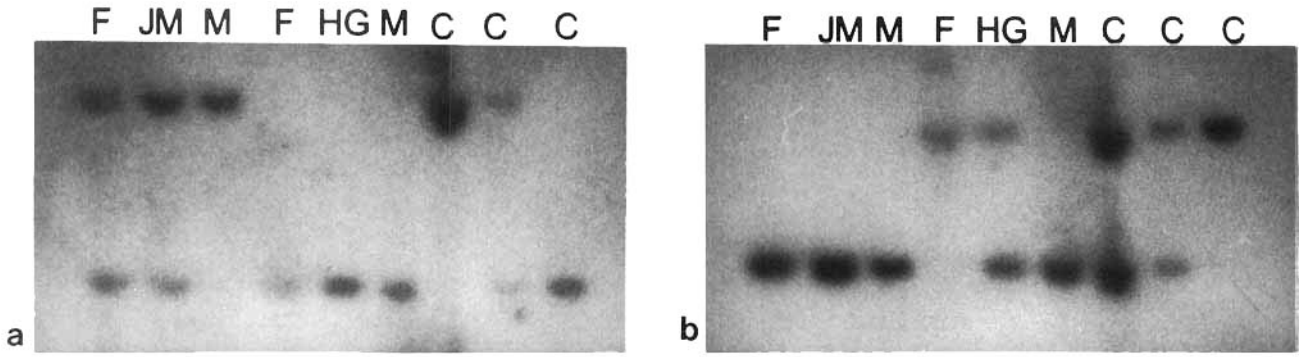

Fig. 3. Detection of duplications using molecular analysis. Genomic DNA from the two patients, their parents, and normal controls was digested with MspI and subjected to Southern analysis. **a:** Probe D17S258. **b:** Probe D17S71. F, father; M, mother; C, control.

sister exchange), or from different mechanisms (unequal sister chromatid exchange in H.G. and unequal crossover in J.M.). Since deletions in SMS may involve either the maternal or paternal chromosome 17, the possibility that the duplications in H.G. and J.M. are of different parental origin is not inconsistent with their duplications being the reciprocal of the SMS deletions.

It is of interest that the SMS region may be the third region on 17p to be involved in duplication/deletion events. The other two regions are the CMT1A/HNPP region [Chance et al., 1994] and the Miller-Dieker (MDS) region in 17p13.3. MDS was first described in 1983 as resulting from a terminal deletion of 17p13.3 [Dobyns et al., 1983]. Recently, Li et al. [1994] reported a case of MDS caused by a duplication of 17p13.3. HNPP is the result of a 1.5 Mb deletion of 17p11.2p12, while CMT1A results from a duplication of the same region [Chance et al., 1994]. It has been proposed that the duplication in CMT1A is due to unequal crossing over between homologous chromosome 17s. Flanking the duplicated/deleted region in HNPP/CMT1A are repeated sequences referred to as CMT1A/REP [Pentao et al., 1992]. These flanking repeats may cause misalignment of the two chromosome homologs during meiosis I, leading to unequal crossing-over and resulting in the two reciprocal cytogenetics events: duplication and deletion.

Regardless of whether the duplication in J.M. involves sister chromatid exchange or crossover, the mechanism is somewhat analogous to the mechanism in HNPP/CMT1A, i.e., unequal alignment of chromatids resulting in a duplication on one chromatid and a deletion on the other. Recent evidence by Chen et al. [1995] suggests that repetitive sequences flank the deleted region in SMS, thus lending credence to the proposal that misalignment of chromatids may occur, as in HNPP/CMT1A, and result in a duplication/deletion event.

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